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Seed coat development, anatomy and scanning electron microscopy of *Harpagophytum procumbens* (Devil's Claw), Pedaliaceae

A. Jordaan*

School for Environmental Sciences and Development, Division Botany, North-West University, Potchefstroom 2520, South Africa

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Abstract

Seed coat development of *Harpagophytum procumbens* (Devil's Claw) and the possible role of the mature seed coat in seed dormancy were studied by light microscopy (LM), transmission electron microscopy (TEM) and environmental scanning electron microscopy (ESEM). Very young ovules of *H. procumbens* have a single thick integument consisting of densely packed thin-walled parenchyma cells that are uniform in shape and size. During later developmental stages the parenchyma cells differentiate into 4 different zones. Zone 1 is the multi-layered inner epidermis of the single integument that eventually develops into a tough impenetrable covering that tightly encloses the embryo. The inner epidermis is delineated on the inside by a few layers of collapsed remnant endosperm cell wall layers and on the outside by remnant cell wall layers of zone 2, also called the middle layer. Together with the inner epidermis these remnant cell wall layers from collapsed cells may contribute towards seed coat impermeability. Zone 2 underneath the inner epidermis consists of large thin-walled parenchyma cells. Zone 3 is the sub-epidermal layers underneath the outer epidermis referred to as a hypodermis and zone 4 is the single outer seed coat epidermal layer. Both zones 3 and 4 develop unusual secondary wall thickenings. The primary cell walls of the outer epidermis and hypodermis disintegrated during the final stages of seed maturation, leaving only a scaffold of these secondary cell wall thickenings. In the mature seed coat the outer fibrillar seed coat consists of the outer epidermis and hypodermis and separates easily to reveal the dense, smooth inner epidermis of the seed coat. Outer epidermal and hypodermal wall thickenings develop over primary pit fields and arise from the deposition of secondary cell wall material in the form of alternative electron dense and electron lucent layers. ESEM studies showed that the outer epidermal and hypodermal seed coat layers are exceptionally hygroscopic. At 100% relative humidity within the ESEM chamber, drops of water readily condense on the seed surface and react in various ways with the seed coat components, resulting in the swelling and expansion of the wall thickenings. The flexible fibrous outer seed coat epidermis and hypodermis may enhance soil seed contact and retention of water, while the inner seed coat epidermis maintains structural and perhaps chemical seed dormancy due to the possible presence of inhibitors.

Keywords: Esem; Secondary cell wall; Seed structure; Ultrastructure; Xerophyte

1. Introduction

Harpagophytum procumbens (Devil's Claw or the grapple plant), is a xerophyte that occurs mainly in the arid, sandy areas of the Northern Cape of South Africa as well as Namibia and Botswana. Fruits of *H. procumbens* have rows of horny arms with recurved spines. Fruits like these with hooks, prickles, or other emergences are typical of members of the Pedaliaceae (Roth, 1977). Ripe fruit on the ground is trampled upon by animals and then the hooked arms adhere so tightly to the feet of animals that it is difficult to dislodge. Animals may stamp the fruits to pieces, whereby the seeds get out of the indehiscent fruit. The mature fruit has 4 locules, and splits longitudinally to release the seeds.

The medicinal properties of Devil's Claw are well known in southern Africa and in the rest of the world. The plant is known to relieve a wide variety of medical disorders such as rheumatoid arthritis due to its anti-inflammatory properties (ESCOP, 2003; Fennell et al., 2004). It is also used for liver function disorders, diabetes, and kidney disorders and for general cleansing of the blood (PharmEur, 2003). The secondary tubers are harvested and

* Tel.: +27 082 804 9789; fax: +27 018 299 2518.
E-mail address: anine.jordaan@nwu.ac.za.

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used to treat various medical conditions. Unsustainable harvesting of tubers has caused the decline of natural populations and due to these practices the plant is considered by some to be endangered (Schneider et al., 2006).

The primary method of natural reproduction in wild populations of Devil's Claw is through seed germination but germination rates are low as the seeds exhibit high levels of dormancy. There is no information available regarding the development of the seed coat of Devil's Claw and very scant information regarding seed development in other members of the Pedaliaceae.

In general, the ultrastructural development of seed coats of southern African xerophytic plants is not well described. Their seed coats may often have peculiar adaptive features such as annular, spiral or variously shaped secondary wall thickenings. Seed coats may also have various appendages like hairs or membranous outgrowths. The function of these thickenings or outgrowths is often related to hydration or dispersal mechanisms (Van Rheede Van Oudtshoorn and Van Rooyen, 1999).

The objective of this study was to investigate seed coat structure and development with light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) in relation to factors that may be associated with seed dormancy and retention of water in an arid environment during seed germination. In an earlier report (Shushu and Jordaan, 2004) attributed the structural basis of seed dormancy to a tight fitting dense multilayered zone, the inner seed coat called the inner skin but further investigation is needed. In this study the inner skin is referred to as the inner seed coat epidermis.

2. Materials and methods

2.1. Light microscopy (LM) and transmission electron microscopy (TEM)

Seeds at different stages of development as well as mature seeds were fixed in Todd's fixative (Todd, 1986). Material was post fixed in 1% OsO_4 in sodium cacodylate buffer, dehydrated in an ethanol series and embedded in LR White resin. Semi-thin (for LM) and ultra-thin (for TEM) sections were cut with a Leica Ultracut R rotary microtome. Semi-thin sections were stained with 0.05% aqueous toluidine blue and neofuchsin and ultra-thin sections were contrasted with 5% aqueous uranyl acetate and lead citrate (Reynolds, 1963) for TEM. Sections were examined with a Zeiss Axioskop II light microscope or Phillips Tecnai 12 TEM.

2.2. Histochemistry

Semi-thin sections of mature seed coats were stained with cresyl violet acetate (CVA) (Keating, 1996) for lignin as well as with ruthenium red for pectic substances (Jensen, 1962).

2.3. Scanning electron microscopy (SEM)

Mature seeds of *H. procumbens* were removed from dry fruits. Seed coats were vapor fixed in OsO_4 and sputter-coated with carbon and gold. Specimens were examined with a Phillips XL30 environmental scanning electron microscope (ESEM) in high vacuum mode. The response of the mature seed coat to moisture was studied using unfixed mature seeds in the ESEM using a Peltier stage.

3. Results

The mature seed coat of Devil's Claw consists of an outer and an inner region. The rough and fibrillar outer region consists of the outer epidermis and hypodermis (Fig. 1a and b). This outer region easily separates to reveal the underlying dense, smooth inner region that is made up by the inner seed coat epidermis that encloses the embryo very tightly. The unusual wall thickenings only develop in the outer seed coat epidermis and hypodermis i.e. the outer region of the seed coat (Fig. 1b).

3.1. Development of the seed coat

A large number of ovules are carried within the 2-loculed ovary at anthesis (Fig. 1c). The placentation is typically axile. After anthesis the false septa enlarge and the 2 locules are closed off to form a 4-loculed ovary (Fig. 1c).

Very young ovules have a single thick integument consisting of densely packed thin-walled parenchyma cells that are uniform in shape and size (Fig. 1d). After anthesis, the nucellus degenerates completely and the inner layers of the integument enlarge and divide periclinally (Fig. 1d). The outer epidermal cells are more or less isodiametric (Fig. 1d). As periclinal divisions continue along the inner layers of the integument the outer layers are crushed and eventually degenerate (Fig. 1e). The outer layers degenerate according to a specific pattern to establish the corrugated appearance of the mature seed coat in surface view (Fig. 1f). The epidermal cells divide anticlinally and further contribute towards the irregular surface pattern of mature seeds (Fig. 1f).

In longitudinal sections the ovule appears hemi-anatropous with the funiculus approximately at right angles with the ovule (Fig. 1g). The funiculus is supplied by a single vascular bundle that terminates in the outer layers of the integument in the region of the chalaza.

During later developmental stages, the initially little-differentiated parenchyma cells that make up the single integument differentiate so that 4 different zones can be seen in the maturing seed coat (Fig. 1h). Zone 1 becomes the multilayered inner seed coat epidermis next to the embryo and has small tangentially elongated cells with no intercellular spaces. Zone 2 becomes the multi-layered middle layer and consists of large irregularly shaped thin-walled parenchyma cells with intercellular spaces that increase in number and size as the cells in this layer degenerate and collapse (Fig. 1h). Zone 3 becomes the outer sub-epidermal layers or hypodermis and consists of more or less isodiametric cells with small intercellular spaces (Fig. 1h). Zone 4 will become the outer epidermal layer of the integument and is composed of large thin-walled cells.

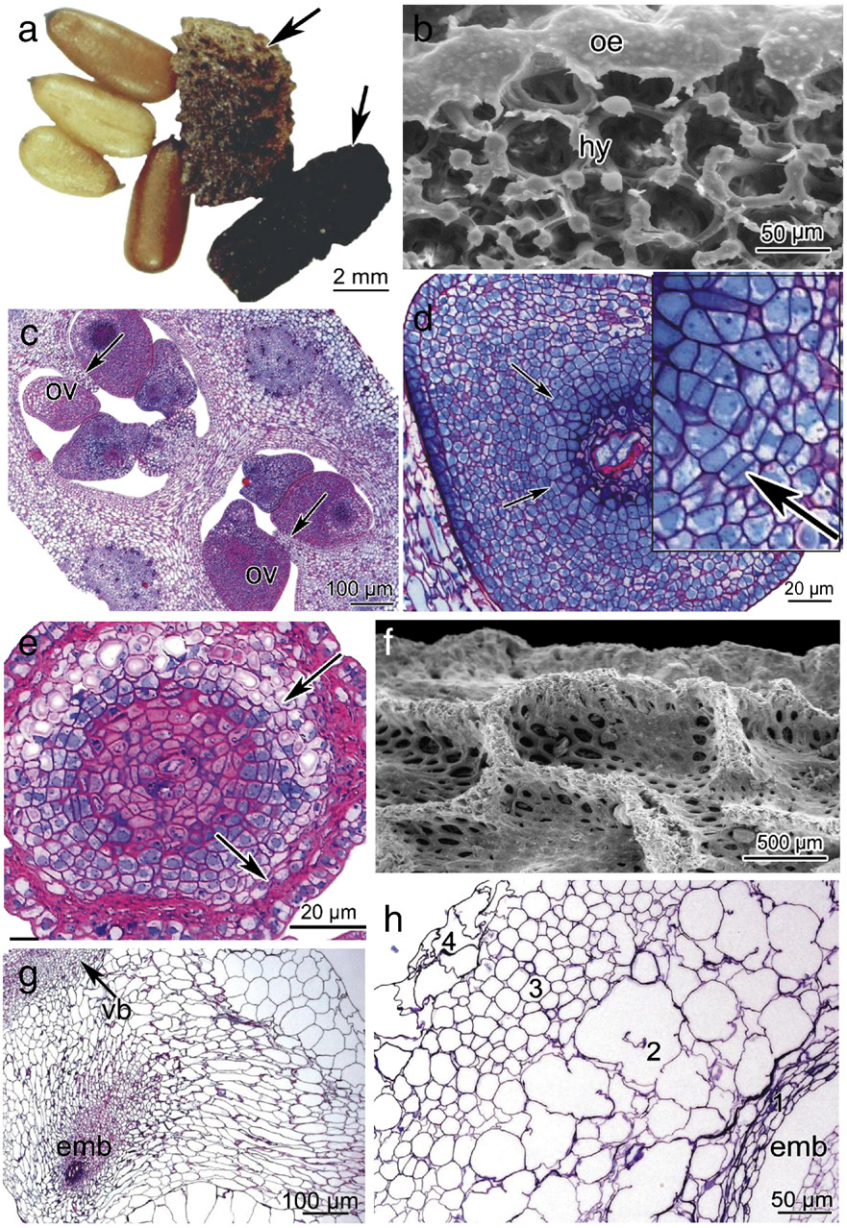

Fig. 1. Mature and developing seeds of *H. procumbens*. (a) Mature seeds with rough fibrillar region of outer seed coat epidermis and hypodermis intact (arrows) and whitish seeds with outer seed coat removed to expose inner seed coat epidermis. (b) SEM micrograph showing mesh-like fibrillar structure of outer seed coat epidermis (oe) and hypodermis (hy) in cross section. (c) Light micrograph of 2-loculed ovary with ovules (ov). Arrows indicate enlarging false septa that will result in 4-loculed ovary. (d) Ovule after anthesis with single thick integument. Arrows indicate periclinal divisions in inner cell layers. (e) Ovule with outer cell layers of integument degenerating (arrows). (f) SEM micrograph of mature seed in surface view showing corrugated appearance of outer seed coat epidermis. (g) Hemi-anatropous ovule in longitudinal section. emb, embryo; vb, vascular bundle. (h) Single integument differentiated into four zones (1–4); emb, embryo.

With further development the inner epidermis (zone 1) of the integument thicken and stain more intensely with toluidine blue (Fig. 2a). Parenchyma cells of the middle layer (zone 2) continue to degenerate so that a space arises between the inner epidermis (zone 1) and the remaining outer layers (Fig. 2a). In effect, this means that connections between the inner epidermis and the outer seed coat zones are severed. No developmental changes were observed in cells of the hypodermis (zone 3) but cells of the outer epidermis (zone 4) enlarge greatly and become radially elongated (Fig. 2a). Eventually, wall thickenings start to develop along the radial walls of the outer epidermis (Fig. 2b). The same type of wall thickenings also develops in the hypodermis but they are not confined to the radial walls and are much smaller (Fig. 2b). The developmental progress of thickenings in both layers are however essentially the same. Wall thickenings in both the outer epidermal and hypodermal layers are initially small and inconspicuous but those in the outer epidermal layer eventually become massive (Fig. 2c).

In the fully differentiated mature seed coat the inner epidermis (zone 1) consists of dense layers of dark-staining tanniniferous cells (Fig 2c). The middle layer (zone 2) consists of large, irregularly shaped degenerating parenchyma cells,

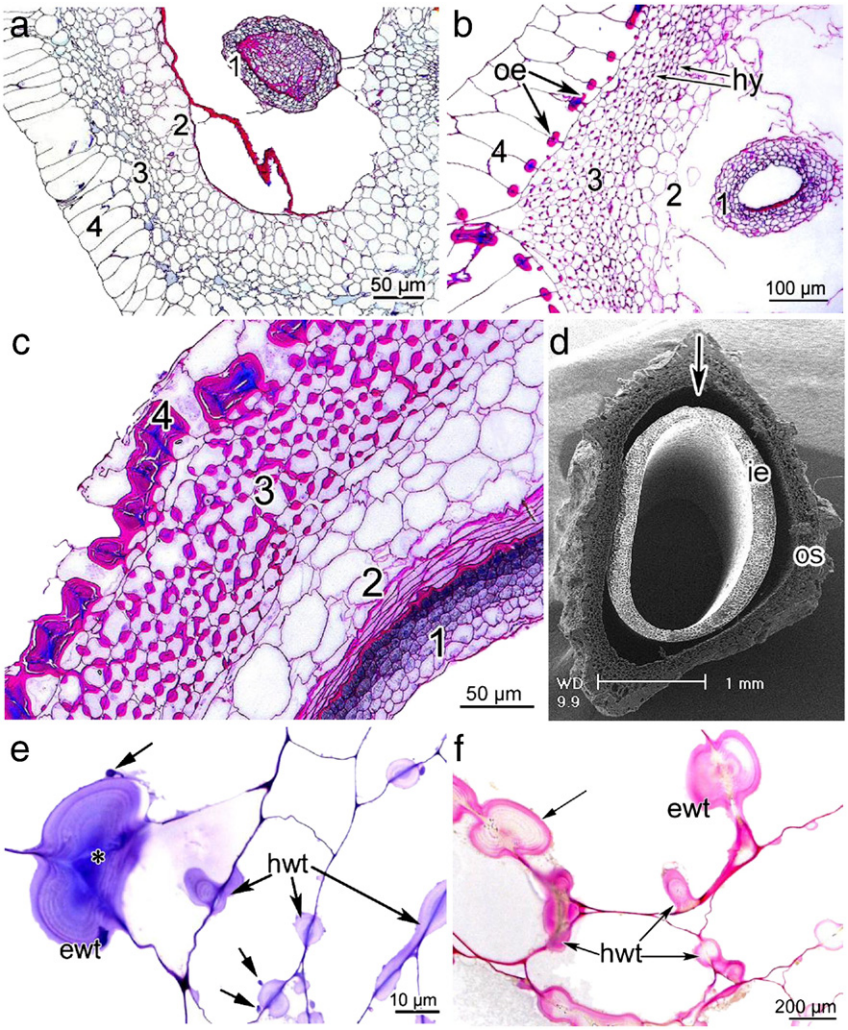

Fig. 2. Seed coat development in *H. procumbens*. (a) Later developmental stage showing degeneration of zone 2, intensely stained zone 1 and radially elongated cells of zone 4 (epidermis). (b) Early stage of wall thickenings developing in outer seed coat epidermis (zone 4—arrows) and hypodermal layers (zone 3—arrows). (c). Fully differentiated mature seed coat with 4 distinctive zones. (d) SEM micrograph of mature seed coat showing inner seed coat epidermis (ie) separated from outer seed coat (os) by a space (arrow) left by disintegrated cells of zone 2. (e) Light micrograph of outer epidermal (ewt) and hypodermal (hwt) wall thickenings in cross section of seed stained with cresyl fast violet. Central part (*) stains more pronouncedly. Arrows indicate large globules associated with wall formation in both types of thickenings. (f) Cross section of seed showing large outer epidermal (ewt) and small hypodermal (hwt) wall thickenings stained with ruthenium red. Arrow indicates more pronounced staining of outer regions of both types of wall thickenings.

some of which are flattened because of massive expansion of the embryo during late embryogenesis (Fig. 2c). With the expansion of the embryo the space seen earlier between the inner epidermis and the middle layer is obliterated, but there are no cytoplasmic connections between the 2 zones. The outer epidermis (zone 4) and the hypodermis (zone 3) have cells with fully-developed secondary wall thickenings (Fig. 2c).

The spatial relationship between the inner seed coat epidermis and the outer seed coat epidermis and hypodermis is clearly visible in SEM cross sections of fully mature seed coats (Fig. 2d). The middle layer has disintegrated completely, leaving a space between the inner epidermis and the hypodermis (Fig. 2d).

The central region of the outer epidermal cell wall thickenings shows a strong reaction for lignin or lignin precursors when stained with cresyl fast violet (Fig. 2e). Globules associated with the formation of the wall thickenings also stain positively, indicating that they may contain lignin precursors (Fig. 2e). The central region of the outer epidermal wall thickenings and globules associated with wall formation stains blue to bluish green with toluidine blue as well, thereby confirming the presence of lignin.

The outer areas of the thickenings show a more distinct staining reaction to ruthenium red than the inner regions, indicating that the outer areas are richer in pectic polysaccharides (Fig. 2f). The contents of vesicles associated with wall formation stain negative, indicating the absence of pectic polysaccharides. The central areas of both epidermal and sub-epidermal wall thickenings also stain negative with ruthenium red (Fig. 2f).

In surface view of fully mature seed coats the outer epidermal and hypodermal secondary wall thickenings assume a 3-dimensional interconnected fibrillar meshwork (Fig. 3a). No intact cells are visible as most of the primary cell walls of the outer epidermis and hypodermis disintegrated during the final stages of seed maturation, leaving only a scaffold of secondary cell wall

thickenings (Fig. 3a). Mature seed coats are characterized by peeling or shedding of some of the primary cell wall material from the wall thickenings in the form of an array of fine tubular or fibrillar structures (Fig. 3b and c). The tubular structures (Fig. 3b) are the former occlusions of the plasmodesmata that attach the secondary wall thickening to the primary cell wall (see ultrastructural formation of wall thickenings).

The multilayered inner seed coat epidermis forms a tough, dense envelope around the embryo (Fig. 3d and e). SEM micrographs indicate that it is a sturdy layer that may mechanically restricts the emergence of the embryo but probably also excludes water and gasses like oxygen required for germination (Fig. 3e). Cells comprising the inner epidermis contain large amounts of dark-staining substances (Fig. 3d), some resembling phenolic compounds. There are also granular dark-staining substances of unknown composition such as inhibitors. The cells of the sturdy layer is thin-walled and do not have any wall thickenings. However the cells are densely and compactly arranged with no intercellular spaces (Fig. 3d). The inner epidermis is delineated on the inside by a few remnant cell wall layers of collapsed nucellar cells that do not stain with uranyl acetate or lead citrate (Fig. 3d and f). On the outside it is delineated by remnant cell walls of collapsed middle layer cells (zone 2) that readily stain with uranyl acetate, and lead citrate (Fig. 3g).

3.2. Formation of wall thickenings

In light micrographs, the first indication of the formation of wall thickenings is the appearance of large globules that stain blue with toluidine blue next to primary cell walls (Fig. 3h). These are the areas where the secondary wall thickenings are about to develop.

Cells where thickenings are about to develop all appear empty and the cytoplasm is confined to a thin layer next to the cell wall (Fig. 3h). Wall thickening formation starts at the plasma membrane, which takes on a more electron dense and uneven outline (Fig. 4a and b). This may be an indication of increased enzyme activity on the plasma membrane. Mitochondria and electron dense globules are present near the developing wall thickening while an abundance of polysomes and ER are present near the plasma membrane (Fig. 4a). Small vesicles containing fibrillar material unite with the plasmalemma followed by an apparent emptying of vesicle contents between the cell wall and plasmalemma (Fig. 4a–c). Large and small vesicles appear to be reservoirs of fibrillar material that are associated with secondary wall thickening formation (Fig. 4c). Vesicles or small vacuoles with fibrillar and membranous contents were present near the developing wall thickenings in the periplasmic space (Fig. 4a) and may therefore be regarded as paramural bodies or lomasomes. Later on, large bodies of fibrillar material were seen close to the developing wall thickenings (Fig. 4c). Deposition of vesicles with fibrillar contents eventually increases and the plasma membrane becomes even more electron dense (Fig. 4c). The small vesicles with fibrillar contents appear to bud off from the large fibrillar body and deposit their fibrillar contents in the periplasmic space (Fig. 4c).

A distinctive ultrastructural feature of the initial stages of wall deposition is the appearance of electron dense globules at regularly spaced intervals along the areas where the secondary wall thickenings are about to develop (Fig. 4d). Closer inspection reveals that the electron dense globules are associated with plasmodesmata (Fig. 4d), hence the reason for their regular spacing. Electron dense material appears to occlude the plasmodesmata prior the commencement of wall development (Fig. 4d and e). Therefore, electron dense non-functional plasmodesmata are the sites where development of the secondary wall thickenings is initiated and where they become attached to the primary walls (Figs. 4e and 5d). Evidence of the occluded plasmodesmata was observed as extensions of electron dense material into the wall thickening itself (Fig. 5d).

Some large cytoplasmic bodies, which may be involved in wall formation, form part of the cytoplasm (Fig. 4f). These large bodies appear to play a major role during later stages of wall formation. They do not fit the description of paramural bodies or lomasomes, instead containing a large consignment of parallel-arranged membranous components, almost resembling etioplasts, together with a fibrillar component (Figs. 4f, 5a, and b).

Different stages of wall thickening development were observed in the same seed coat. One thickening may be complete while an adjacent one is initiated or still in the process of being formed with vesicles adhering to the outermost layer of the wall thickening (Fig. 5b and d). Electron dense and electron transparent layers with central electron dense areas characterize fully formed wall thickenings. This layering is also conspicuous in light and SEM micrographs (Fig. 5e and f).

Fig. 5c and d illustrates the electron dense and electron lucent regions of a developing wall thickening. An electron dense plasma membrane occurs next to the surface of the fibrillar amorphous region of the wall thickening. Next to this electron dense plasma membrane is another plasma membrane that is less electron dense (Fig. 5c).

Although wall thickenings of the outer epidermis are more massive than those of the hypodermis, the ultrastructural sequence of wall formation is essentially the same (Fig. 5e and f). Wall layering is however more conspicuous in the outer epidermal wall thickenings. In the mature seed coat of Devil's Claw the thin primary cell walls in the epidermal and sub-epidermal layers eventually disintegrate (Fig. 2c).

3.3. ESEM studies

On the outer surface of the seed coat the disintegration of the primary cell walls can be seen as a peeling away in the form of separate or continuous sheets of fine fibrils from the secondary wall thickenings (Fig. 6a). These fibrils are hygroscopic and as they easily imbibe water, entire sheets of them expand and coil up (Fig. 6b). There are also what appears to be cytoplasmic remnants of small, irregularly shaped particles (Fig. 6c) on the surface of the wall thickenings.

The middle lamellae together with portions of the primary cell walls are present in the form of ridges between the wall

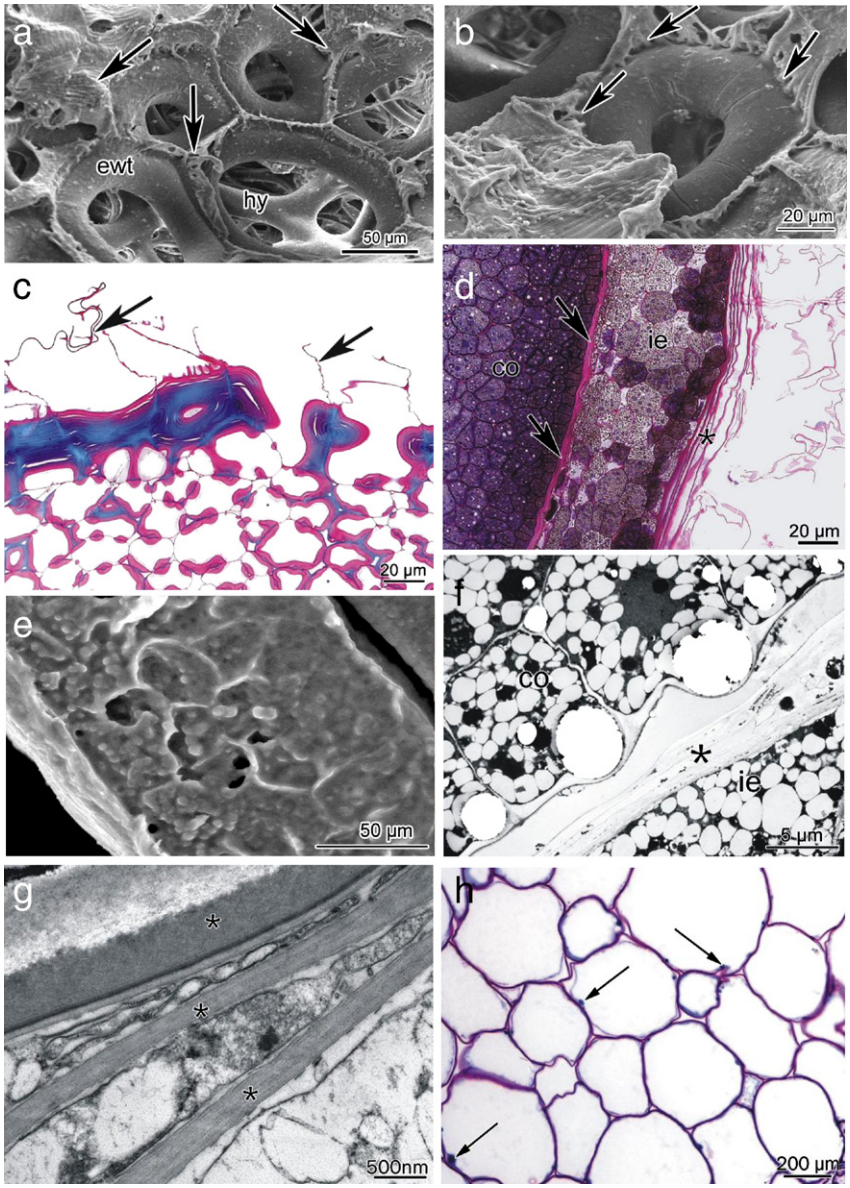

Fig. 3. Light and electron micrographs of different zones of developing and mature seed coat. (a) SEM micrograph of mature seed coat in surface view with epidermal wall thickenings (ewt) and hypodermal wall thickenings (hy) underneath. Arrows indicate remnants of primary cell walls. (b) Outer epidermal wall thickening showing former plasmodesmata in the primary cell wall attached as tube-like remnants (arrows) to the secondary cell wall of thickening. (c) Light micrograph showing disintegrating primary cell walls (arrows) in outer seed coat epidermis. (d) Light micrograph of inner seed coat epidermis (ie) containing dark-staining substances and bordered on the inside by remnant cell wall layers of nucellus (arrows) and of zone 2 (*) on the outside. (e) SEM micrograph of inner seed coat epidermis showing densely packed cells. (f) TEM micrograph of remnant cell wall layers (*) of the nucellus bordering inner seed coat epidermis (ie) on the inside, weakly stained with uranyl acetate and lead citrate. co, cotyledons. (g) Remnant cell wall layers of zone 2 (*) bordering inner seed coat epidermis on the outside, strongly stained with uranyl acetate and lead citrate. (h) Light micrograph of first stage of development of hypodermal wall thickenings in zone 3. Arrows point to globules where thickenings are about to develop.

thickenings (Fig. 6e and f). As these ridges become wet they expand and becomes like a jel, flowing and forming very thin sheets over the cell lumina (Fig. 6e) of the outer epidermis or they may form thick viscous layers that occlude the lumina of the wall thickenings (Fig. 6f).

4. Discussion

When germination occurs in arid and semi-arid environments seeds must be able to take up and retain moisture for successful imbibition. Many desert species have special anatomical and ultrastructural adaptations for moisture retention (Fahn and Cutler, 1992). Pectic substances are known to ease imbibition and assist in the retention of substantial quantities of water in some xerophytes (Lyshede, 1978, 1984). In *H. procumbens* it was evident from histochemical tests that the outer cell wall ridges that absorbed water and started to flow when wet were rich in pectic substances. This observation is supported by Ernst et al. (1988) who calculated that seeds of *H. procumbens* were able to increase weight by more than 250%

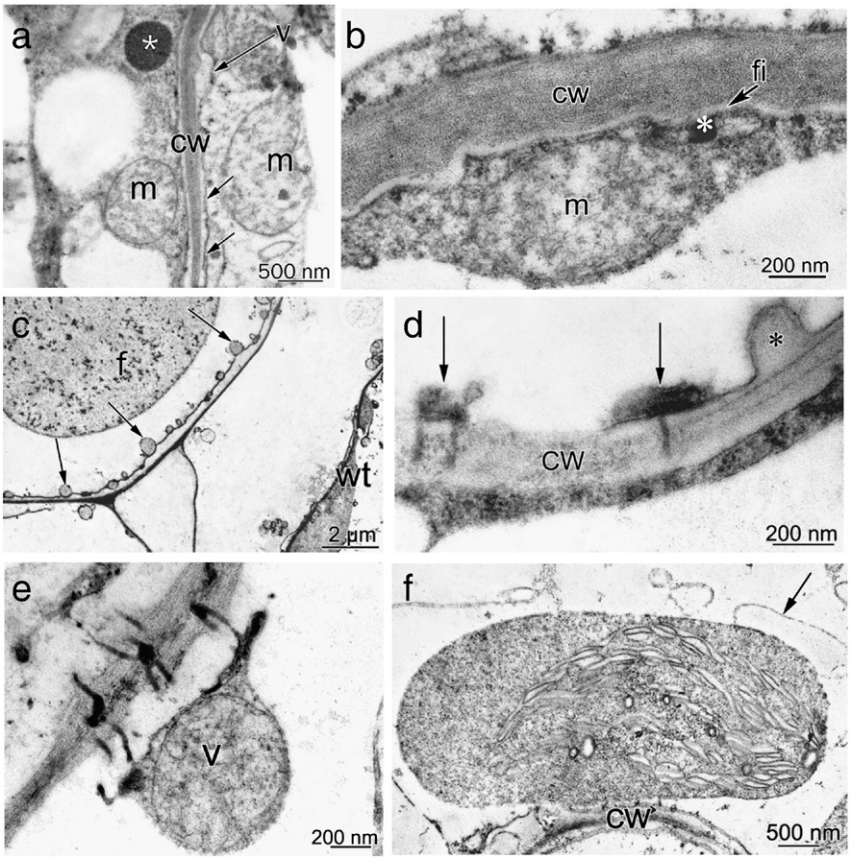

Fig. 4. TEM micrographs of first ultrastructural events associated with developing wall thickenings. (a) Electron dense plasma membrane (arrows), possible fusion of vesicle (v) or paramural body with plasma membrane and discharge of fibrillar material (fi) in periplasmic space. cw, cell wall; m, mitochondria, * indicates electron dense globules. (b) Fibrillar material (fi) between plasma membrane and cell wall (cw), electron dense globules (*) and mitochondria (m) in region of developing wall thickening. (c) Large numbers of vesicle (arrows) with fibrillar contents budding of from large fibrillar body (f) and fusing with electron dense plasma membrane. wt, wall thickening in adjacent cell. Large fibrillar body (f) corresponds to globule seen in light micrograph of Fig. 3h. (d) Electron dense plasmodesmal occlusions (arrows) where wall thickenings are about to commence development. (*) indicates fusion of vesicle with plasma membrane and discharge of fibrillar contents between plasma membrane and cell wall. (e) Electron dense strands in plasmodesmata and adjacent vesicle (v). Note swollen primary cell wall. (f) Large cytoplasmic bodies with fibrillar and membranous (arrows) components involved in the formation of wall thickenings.

after a short imbibition period of only 30 min. Frey-Wyssling (1976) mentioned that the pectin of the middle lamellae and primary cell wall was insoluble but might have played a substantial role in imbibition as it swelled significantly when wet and shrank when dry. Ruthenium red is not a reliable dye for pectin, it does however give an indication of the distribution of pectic polysaccharides in the wall thickenings. Contrary to expectation the strongest staining reaction was in the outer layers of the wall thickenings and not the central wall areas as would be expected where the pectin-rich middle lamellae and primary cell wall abuts the secondary wall thickenings.

The inner seed coat epidermis may be involved in structural and possibly chemical dormancy. Experiments by Ernst et al. (1988) revealed that the seed coat of *H. procumbens* is a major barrier to full imbibition of the embryo and endosperm. The compressed remnant cell wall layers of the nucellus on the inside of the inner epidermis and of the middle layer on the outside may contribute towards impermeability. This may especially concern the inner compressed cell wall layers of the nucellus that did not stain with uranyl acetate. According to Werker (1997) in some species, remnant cell wall layers of the integument may be impregnated with phenols or these compressed layers may form a superficial "pelicle" on the seed surface. It is common to form deep-seated pellicle layers as well from other cell layers of the integument.

The presence of phenolic compounds and other dark-staining substances in the inner epidermis may contribute towards chemical dormancy. Phenolic compounds are regarded as oxidizing agents thus binding to oxygen and preventing it from being available to the embryo (Côme and Tissaoui, 1973).

Without an anatomical investigation of the seed coat structure, it is easy to assume that the outer epidermis and hypodermis that easily detach from the remainder of the seed coat constitutes the entire seed coat. Ernst et al. (1988) suggested that the endosperm may act as a mechanical barrier to water uptake but he may unintentionally have referred to the inner seed coat epidermis as the endosperm. Obviously, the inner epidermis encloses the endosperm very tightly and cannot be removed. The part that was removed by Ernst et al. (1988) prior to experimentation was probably only the outer seed coat.

It is common for related members of the Pedaliaceae such as *Sesamum* and *Proboscidea* to display powerful seed dormancy

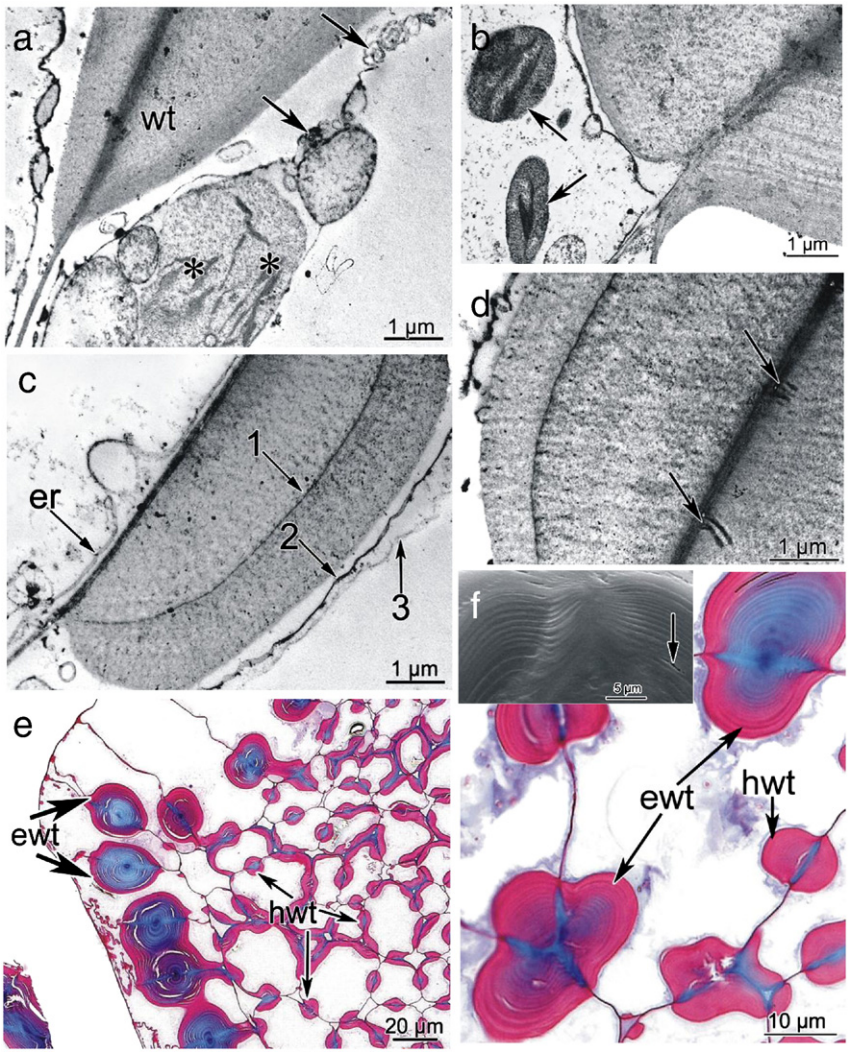

Fig. 5. Light and electron micrographs of final stages of wall thickening formation. (a) Large cytoplasmic body with fibrillar and membranous (*) components near almost mature wall thickening (wt). Arrows indicate "unraveling" of membranous component. (b) Similar, but smaller cytoplasmic bodies near almost mature wall thickening. (c) Electron dense plasma membrane already deposited into wall thickening (arrow 1) and another plasma membrane (arrow 2) about to be deposited on the surface of the wall thickening. Arrow 3 indicates formation of new plasma membrane. (d) Almost mature wall thickening with electron dense material in occluded plasmodesmata (arrows) that anchor wall thickenings to primary wall. (e) Light micrograph of mature outer fibrous seed coat with outer epidermal (ewt) and hypodermal (hwt) wall thickenings stained with toluidine blue and neofuchsin. Note that the central areas of the thickenings show a distinctly different staining reaction than the outer regions. Primary cell walls are still intact. (f) The layered appearance of almost mature epidermal wall thickenings (ewt) is conspicuous in light- and scanning electron micrographs (insert). The arrow indicates a space where their is a separation of lamellae. The layering of hypodermal wall thickenings (hwt) are less obvious.

due to chemical inhibitors (Bedigian et al., 1985; Nabhan et al., 2000). However, unlike *H. procumbens*, the seeds germinate profusely after abundant rainfall, suggesting that the inhibitors easily leach out of the seeds. It is unlikely that the outer epidermis and hypodermis of *H. procumbens* retain enough moisture to flush inhibitors from the inner epidermis. Also all the connections between the inner epidermis and outer seed coat layers (outer epidermis and hypodermis) are severed during early developmental stages. This excludes the possibility that the wall thickenings of the outer seed coat may act as one way channels or valves that conduct water to the inner seed coat or even to the embryo and endosperm.

The seed coat wall thickenings of Devil's Claw shows structural similarities with the fruit capsule of *Hemimeris montana* a member of the Scrophulariaceae that occurs in the arid regions of Namaqualand (Van Rheede Van Oudtshoorn and Van Rooyen, 1999). Upon hydration, the entire capsule of this plant expands considerably to liberate the seeds. This is an example of hydrochasy where fruit opening is induced by wet conditions, a phenomenon common in desert plants (Gutterman, 1993). The seed coat of *H. procumbens* shows similarities with the hydrochastic tissues of desert fruits as it expands when wet, primarily due to hydrophilic cytoplasmic remnants and remnants of the primary cell wall and middle lamellae.

It has been reported that seeds of *H. procumbens* are released very slowly from the fruit (Stewart and Cole, 2005) and Ernst et al. (1988) calculated that only about 25% of seeds are released into the soil annually. Nevertheless, even when the

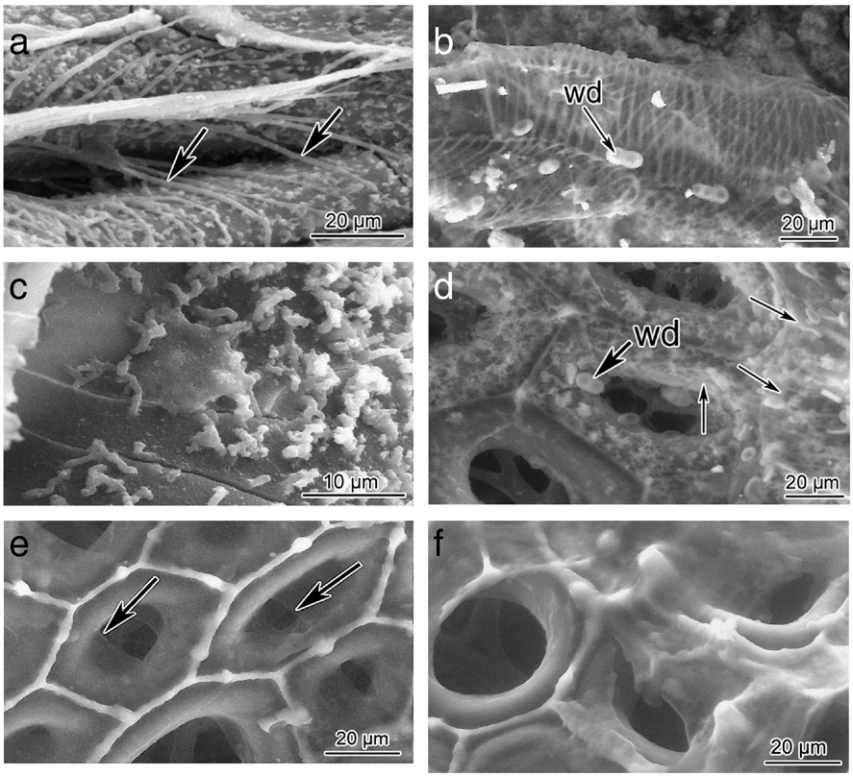

Fig. 6. SEM micrographs of the surface of mature seed coat of *H. procumbens* in the dry and wet state. (a) Outer surface of the fibrillar seed coat in the dry state with detaching fine fibrils (f) from primary cell walls and irregularly shaped particles of cytoplasmic remnants. (b) Detaching fine fibrils (f) from primary cell wall showing expansion and coiling after 30 min imbibition. wd, water droplets. (c) Particles of cytoplasmic remnants expanding (arrows) as it reacts with water after 30 min imbibition. (d) Cytoplasmic remnants absorbing water and forming homogenous layers (arrows) after 45 min imbibition. (e) Middle lamellae and primary cell walls becoming jel-like after imbibition and forming thin sheets (arrows) over the cell lumens of the outer seed coat layer after 1 h imbibition. (f) Advanced stage of imbibition after 2 h with thick viscous layers forming due to jellation of primary cell walls and flowing of ctoplasmic remains over cell lumens.

fruits are partially open the seeds are very closely packed and difficult to dislodge mechanically (Jordaan, personal observation). Wet and dry cycles may however have the effect of dislodging the seeds. While still inside the fruit the fibrous seed coats may swell when wet and shrink when dry. The constant swelling and shrinking of seed coats may enlarge spaces between seeds and cause the outer seeds to drop out of the fruit more easily.

The wall thickenings of Devil's Claw resemble the structure of a lamellate type of tilosome found in the velamen of certain groups of the Orchidaceae (Figueroa et al., 2007). Therefore, the possibility that the wall thickenings are involved in hydration and retention of water is indeed plausible as they are appropriately hygroscopic. In some instances, the velamen of canopy-dwelling orchids may be extremely hygroscopic, allowing it to become engorged with water within seconds (Benzing et al., 1982). ESEM observations confirmed that the outer seed coat of *H. procumbens* is hygroscopic despite the presence of lignin. Similarly, Pridgeon et al. (1983) mentioned that the excrescences of the innermost velamen layer called tilosomes are also lignified in many epiphytic orchids but this does not seem to impede their function of water uptake.

The ultrastructural development of the wall thickenings show similarities with those of tracheids, vessel members (Cronshaw and Bouck, 1965), endodermal wall thickenings (Schreiber et al., 1999) and the thickenings found in the velamen of orchids (Moreiro and Dos Santos Isaias, 2008). All these types of secondary wall thickenings develop over primary pit fields. Cytologically thin layers of parietal cytoplasm and abundance of vesicles with fibrillar or globular electron dense contents are associated with their development. The highly electron dense plasma membrane is also characteristic of the formation of secondary wall thickenings in vessel members, indicating that enzymatic reactions occur on the plasma membrane as wall precursors are modified to be incorporated into the wall (O'Brien, 1972).

It is especially common for wall thickenings of xylem elements to develop over plasmodesmata (Cronshaw and Bouck, 1965). According to Kragler et al. (1998) this phenomenon is especially conspicuous in tracheary elements because the plasmodesmatal connections between the developing tracheary element and xylem parenchyma is severed by the deposition of wall material over the plasmodesmata. This is in order to seal the plasmodesmata to facilitate programmed cell death of the tracheary elements (Lachaud and Maurousset, 1996).

Vesicles and other membranous structures in the space between the plasma lemma and developing wall thickenings resemble paramural bodies or lomasomes (Hall et al., 1982) or even "lamellar bodies" a term used previously to describe a

membrane reservoir (Hodge et al., 1956), More recently the term multivesicular bodies (An et al., 2007) was ascribed to some of these membranous structures. The term paramural body or lomasome is however reserved for membranous bodies in the periplasmic space and these are thought to be associated with wall synthesis (Marchant and Robards, 1968; Chaffey, 1996). The membranous bodies in the cytoplasm are not paramural bodies or lomasomes because of their position outside the periplasmic space. Neither are they lamellar bodies or multivesicular bodies because they look different. Nevertheless they appear to be associated with wall formation as they "unravel" there membrane component as templates for the many appositions of the wall thickenings. There primary role therefore appears to act as membrane reservoirs.

Myers et al. (1956) first suggested the possibility that part of the plasma membrane may be incorporated into the wall. Cronshaw and Bouck (1965) did not directly observe this phenomenon in wall thickening of xylem elements but speculated that a clear area inside the plasma membrane may indicate that a new plasma membrane is about to be deposited, together with a region of the cytoplasm into the wall. This appears to correspond to wall layer deposition in *H. procumbens*. The banded appearance of the wall thickenings in *H. procumbens* are due to electron lucent fibrillar regions and electron dense areas. Cronshaw and Bouck (1965) attributed the banded appearance of secondary wall thickenings of xylem elements to fixation and staining of the enzyme system concerned with lignification or to lignin itself.

5. Conclusion

Many seeds that have to survive the unpredictable and unfavourable conditions of arid and semi-arid regions follow the "safe strategy", germinating only after sufficiently large amounts of rain have fallen (Gutterman, 1993). This study indicates that *H. procumbens* follows this strategy, germinating only when adequate rain showers have washed inhibitors out of the inner seed coat epidermis. This strategy ensures that the chances of seedling establishment is very high and the risks very low. These types of seeds are usually well protected from extreme environmental conditions and this is often reflected in their interesting anatomical structure.

This study showed that the seed coat of *H. procumbens* is derived from only one integument. The integument has 4 identifiable zones but the mature seed coat only has 3 zones due to the disintegration of zone 2 or the middle layer. The inner epidermis is distinct from the outer epidermis and hypodermis due to the absence of wall thickenings. The inner seed coat epidermis of *H. procumbens* may not only impede penetration of water and oxygen but may also physically restrain the emergence of the radicle. Due to the dense arrangement of cells and the presence of tannins, lipids and various other unidentified substances this layer is probably the cause of seed dormancy. Scarification of the inner epidermis did improve germination, but not significantly (Shushu and Jordaan, 2004). This suggests that the tannins and other unidentified substances may play a significant role in chemical seed dormancy and needs further investigation.

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